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TITLE: Development of Antibodies Against Novel Cell Surface Proteins in Hormone Refractory Prostate Cancer

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14. ABSTRACT The overall goal of this project is to validate novel targets and develop new antibody reagents for the diagnostic imaging and treatment of prostate cancer. N-cadherin is a cell surface marker that is overexpressed in hormone refractory prostate cancer which could have possible diagnostic or therapeutic utility. We observed that the cadherin profiles of certain prostate cancer cell lines and xenografts correlated with their level of invasiveness and that amongst certain hormone refractory xenograft models, there was a consistent upregulation of N-Cadherin when compared to its androgen dependent counterpart. We sought to validate N-Cadherin as a target in hormone refractory prostate cancer and determine if blockade of this pathway could decrease invasion and metastasis. We generated specific monoclonal antibodies against different domains to determine if blockade could decrease invasion and metastasis. Ongoing in vitro and in vivo studies will determine if this protein can be validated as an effective imaging and therapeutic target.					
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INTRODUCTION

The process known as Epithelial-Mesenchymal Transition (EMT) is becoming increasingly implicated as a crucial mediator of carcinogenesis. This phenomenon is manifested by “Cadherin Switching” in which Epithelial (E) Cadherin is lost and Neuronal (N) Cadherin is upregulated. In preliminary studies, we observed that the cadherin profiles of certain prostate cancer cell lines and xenografts correlated with their level of invasiveness. Preliminary evidence suggested that blocking the switch from E-Cadherin to N-Cadherin may block motility and invasiveness of prostate cells in vitro. We sought to validate N-Cadherin as a target for diagnostics of therapeutic purposes in hormone refractory prostate cancer and determine if blockade of this pathway could decrease invasion and metastasis.

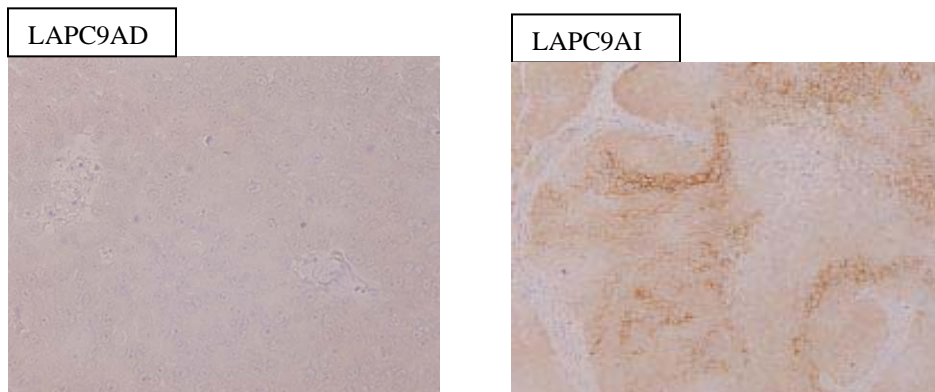
PROGRESS REPORT

Aim 1: Target validation

Subaim 1.2: Correlation with clinical outcomes

Staining conditions for N-cadherin antibody are being optimized. Initial staining on xenografts appears consistently reproducible. Staining with RNA in-situ hybridization is ongoing.

*Immunohistochemical staining with N-cadherin antibody
is membrane specific in hormone refractory xenografts*



Subaim 1.3: Role of N-cadherin in prostate cancer

We have constructed lentiviral constructs when tested in cells that overexpress N-cadherin. We have three prostate cancer cell lines (MDA, LAPC4, and LNCAP) that overexpress N-cadherin and are testing one of the lines for tumorigenicity and progression to androgen independence in non-castrate and castrate mice. We ran into some technical difficulties in the generation of the cell lines, specifically the virus stock. These generated lines are being tested in vitro using the Boyden chambers to measure motility and invasion (Matrigel coated). In addition, we are testing invasion and motility using our N-cadherin antibodies (see below).

Aim 2: Antibody development

Subaim 2.1: Recombinant N-cadherin

We generated separate N-cadherin constructs, one containing the entire extracellular domain (1-5) and one containing only the fourth extracellular domain that is thought to be the most critical for invasion and motility.

Subaim 2.2: Antibody development using a hybridoma strategy

As outlined in the grant, hybridomas for both the N-cadherin antibodies were produced at Caltech and screened by ELISA against recombinant N-cadherin. Three clones were found and characterized for the intact N-cadherin. Two of the clones are the same antibody, but one clone 1H7 recognizes a different binding site. Two clones for the EC4 domain were also characterized.

Subaim 2.3: Isolation and characterization of phage antibodies

We also have begun developing monoclonal antibodies against N-Cadherin using a human phage display library which will allow us to obtain single chain Fv fragments that can then be used for in vivo imaging testing. This process is ongoing.

Aim 3: Preclinical Testing

Subaim 3.1: Intact fully human antibodies for in vitro and in vivo pre-clinical testing

Cell lines that reflected high levels of N-cadherin were antibody treated with the specific monoclonal N-cadherin antibodies against the different domains at various concentrations. Proliferation and invasion of these cells were checked by MTT and Boyden invasion chambers. Expression levels of EMT-associated factors were measured by Western blotting and compared in treated and untreated cells.

In all high-N-Cadherin expressing cell lines, antibody treated cells demonstrated phenotypic changes consistent with a reversal of the EMT (improved polarity, less fibroblastic appearing). Inhibition of N-Cadherin using our monoclonal antibody also resulted in about 50% decreased invasion in prostate cancer cell lines when compared to untreated cells ($p < 0.001$). No appreciable differences were seen in the rates of proliferation. Western blotting revealed that some N-Cadherin positive, E-Cadherin negative cell lines expressed E-Cadherin after prolonged treatment with the antibody suggesting a reversion of the EMT.

Anti-N-Cadherin monoclonal antibodies changes cellular morphology in PC3 cells

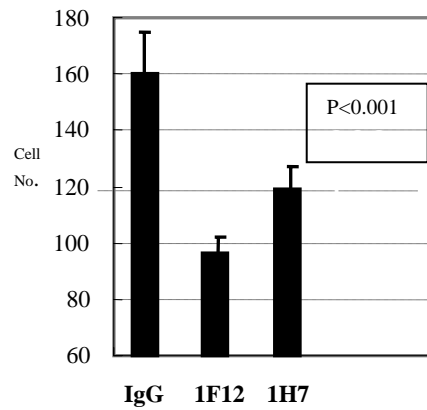
Antibody treated cells



Control



Invasion assay with anti-N-Cadherin monoclonal antibodies in PC3 cells



Subaim 3.2: In vivo testing of progression to androgen independence

We observed that amongst certain hormone refractory xenograft models, there was a consistent upregulation of N-Cadherin when compared to its androgen dependent counterpart. Our plan is to proceed with in vivo testing to determine if the blockade of N-Cadherin can delay the progression to androgen independence.

KEY RESEARCH ACCOMPLISHMENTS

- Developed and affinity purified an intact monoclonal anti-N-cadherin antibody and one containing only the fourth extracellular domain. These antibodies have been tested in various in vitro experiments and are now well validated.
- Observed phenotypic changes in anti-N-cadherin treated cells consistent with a reversal of the EMT.
- Observed a decrease in invasion by antibody treated cells compared to untreated controls.
- Able to detect signal in immunohistochemistry control samples using our affinity purified monoclonal anti-N-cadherin antibody.

REPORTABLE OUTCOMES

None to date.

CONCLUSION

We have developed and purified several monoclonal anti-N-cadherin antibodies that have been validated both by immunohistochemistry (with positive and negative controls) as well as with invasion assays. Blockade of N-Cadherin by several monoclonal antibodies results in a more polarized phenotype by decreasing invasion in both bladder and prostate cancer cell lines. Reversal of this EMT may restore tumors to a more polarized and epithelial phenotype. Ongoing studies are examining the effects of these anti-N-Cadherin antibodies in xenograft and orthotopic mouse models.

We have encountered difficulties in developing a monoclonal anti-Ly6E antibody due to the insolubility of the fusion protein and its high cysteine content. In addition, RNA in situ hybridization using sense and anti-sense probes of Ly6E have not been fully analyzed. We are continuing to determine if this protein is validated by testing the RNA probes in a new array. This will allow us to assess whether or not further targeting of this protein is warranted.

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